

Degradation of Oil Shale by Sulfur-Oxidizing Bacteria

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Approximately 40% of oil shale can be solubilized by the action of sulfur-oxidizing bacteria. *Thiobacillus thiooxidans* and *Thiobacillus concretivorus* are equally effective in solubilization. Continuous leaching experiments show that this process can be completed within 14 days. The growth of *Thiobacillus* and the production of acid were measured under several conditions. Almost all of the $\text{CaMg}(\text{CO}_3)_2$ was removed by this process, leaving a complex of silica and kerogen that could be burned as low-energy fuel. The silica-kerogen complex had not yet been biologically degraded.

The purpose of this research was to determine whether the sulfur-oxidizing bacteria could be used to release kerogen and bitumen, the predecessors of shale oil, from oil shale. Oil shale is an energy source of tremendous potential importance, and the estimates of the abundance of this fossil energy source indicate that it is many times greater than all of the petroleum deposits of the United States.

The component of oil shale of the greatest use as an energy source is kerogen which is complexed with dolomite (magnesium-calcium carbonate) and with quartz. The present use of oil shale deposits is hindered by the difficulty in separating the usable organic portion from the mineral matrix. This process involves retorting, which uses a large amount of energy and only incompletely liberates the trapped organic matter.

If a large fraction of the mineral matrix could be destroyed readily, such as the rapid dissolution of the dolomite portion of the matrix or the splitting of the silica linkages through the action of sulfuric acid produced by *Thiobacillus* species, the recovery of the organic portion would then become feasible.

MATERIALS AND METHODS

Medium. *Thiobacillus* cultures were grown on a medium containing: $(\text{NH}_4)_2\text{SO}_4$ (0.2 g); KH_2PO_4 (3.0 g); $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (0.5 g); $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (0.25 g), and distilled water (1 liter). The medium was adjusted to pH 3.5 with 10 N H_3PO_4 . Elemental sulfur (Mallinckrodt, St. Louis, Mo.) was layered on the surface of the medium before sterilization by intermittent steaming on 3 consecutive days for 30 min. Autoclaving was not used since it fuses sulfur into a solid agglomerate.

Preparation of cultures. *Thiobacillus thiooxidans*

ATCC 8085 and *T. concretivorus* ATCC 19703 were obtained from the American Type Culture Collection. The standard inoculum was 10 ml of a 7-day culture per liter of fresh medium. Cultures were maintained at room temperature without shaking in Fernbach flasks presenting a large surface-to-volume ratio.

Oil shale. The oil shale used in these experiments was from the Green River area of the Mahogany Ridge series. It was supplied by G. U. Dinneen of the Bureau of Mines.

The whole-rock percentages of carbonate were determined by using a Leco gasometric analyzer (7). The shale samples were ground and sieved to obtain a size passed by a Tyler standard screen scale no. 16 and retained by a no. 42.

Green River oil shale consists of approximately 86% mineral and 14% organic material. Mineralogically this shale is a fine-grained, varved, calcareous, sedimentary rock. Varves consist of layers of carbonates and orthoclase. Clay and organic material are intimately associated. The mineralogy of a typical Green River oil shale shows quartz (SiO_2) and dolomite [$\text{CaMg}(\text{CO}_3)_2$] to be the major mineral constituents. Montmorillonite is the most abundant clay mineral and accounts for about 15% of the sample weight. Pyrite is also present at approximately a 2.0% level.

The organic material contained in oil shale consists of bitumen and kerogen. Bitumens, a minor constituent, may be extracted with organic solvents. The organic material remaining in shale after extraction is kerogen. This is defined as the indigenous material in fine-textured sedimentary rock, insoluble in organic solvents, and from which oil can be obtained by heating at 500 C (3).

The chemical characterization of kerogen from Green River shale was carried out after removal of the carbonates by solubilization with hydrochloric acid and removal of the silica and silicate with hydrofluoric acid. The kerogen molecule is a large network of hydrocarbon components with highly cross-linked structures which is largely saturated and contains some heterocyclic bridges.

Leaching experiments. In experiments involving leaching of shale by cultures, acid solution, or acid solution plus an additive, 50 g of shale was placed in a cylinder (50 by 200 mm) with a stopcock on one end. The solution was allowed to percolate down through the shale at a rate of 1 liter per day. The shale was immersed continuously in the leaching solution. The pH of the cultures used for leaching was 1.7 to 1.9. Unless otherwise indicated, the leaching experiments were carried out for 14 days. At the end of the leaching period, the shale was quantitatively recovered and dried for 24 h under an infrared lamp.

Analytical methods. Sulfate determinations were made by adding 4 drops of 6 N HCl to a 10-ml volume of membrane-filtered sample followed by 0.5 ml of a saturated solution of barium chloride. The precipitate was quantitatively collected and dried to constant weight.

Ferrous ion was determined spectrophotometrically after reaction with 1,10-phenanthroline (4).

RESULTS

Stationary cultures. Cultures of *T. thiooxidans* and *T. concretivorans* were grown on inorganic salt medium with elemental sulfur as an energy source and atmospheric CO₂ as a carbon source. Growth curves for both microorganisms in this medium are presented in Fig. 1.

The acid production during development of the culture resulted in a decrease in pH in the weakly buffered medium. *T. thiooxidans* inoculated into inorganic sulfur medium at an initial pH of 3.5 resulted in a decrease in pH (Fig. 2, solid circles). Inorganic sulfur medium with 20 g of shale per liter showed the pattern of pH for the first 4 days after inoculation. The initial

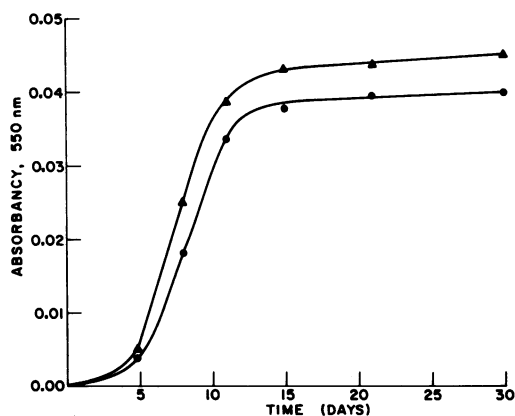


FIG. 1. Growth curve measured by increase in turbidity at 550 nm for stationary cultures of *T. thiooxidans* (●) and *T. concretivorans* (▲). Cultures were grown in inorganic salt medium with 10 g per liter of elemental sulfur suspended on the surface. One liter quantities were dispersed in 2.8-liter Fernbach flasks with a large surface-to-volume ratio.

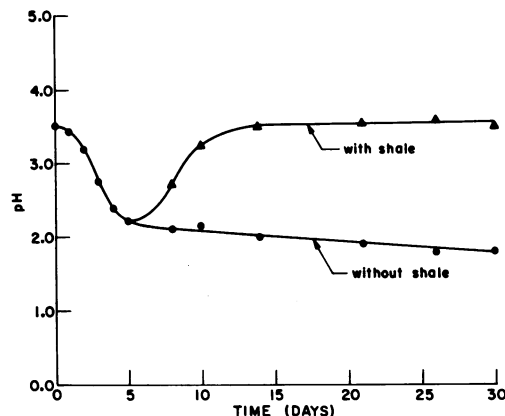


FIG. 2. Change in pH during growth of *T. thiooxidans* in inorganic salt medium with elemental sulfur as the energy source. Volume of the culture was 1 liter and the cultures were incubated without oil shale. Culture with 20 g per liter of Tyler standard screen mesh shale (no. 16 to no. 42) (▲); ●, *T. thiooxidans* inoculated into inorganic sulfur medium at an initial pH 3.5.

decrease in pH was followed by an increase (solid triangles) as a result of buffering by carbonates released from the shale.

Shale was hydrophobic as indicated by the tendency of the ground shale particles to clump together and resist wetting. Shale was wetted by the culture fluid after the culture developed turbidity. Wetting of the shale was indicated by dispersion of shale particles as well as the precipitation of the floating sulfur. Precipitation of sulfur is known to result from the production of a wetting agent, phosphatidyl inositol, by the growing *Thiobacillus* culture (6).

The amount of sulfate produced in stationary *T. thiooxidans* cultures with 20 g of shale per liter was similar to the amount of sulfate produced in cultures containing no shale (Table 1). This indicates that the metabolism was neither inhibited nor stimulated by inclusion of shale at this level in this medium.

Shale recovered from stationary *T. thiooxidans* cultures incubated for 30 days was found to have lost an average of 12.4% in weight. *T. concretivorans* cultures grown under similar conditions gave an average weight loss of 15.1%.

Leaching of shale. Fourteen liters of a 42-day culture of *Thiobacillus* (pH 1.7 to 1.9) was percolated through 50 g of oil shale at the rate of 1 liter per day. (At 42 days, the maximum concentration of acid was present.) The pH of the effluent from the leaching columns was monitored (Fig. 3). The pH of the effluent from

TABLE 1. Sulfate production in inorganic sulfur medium^a

Organism	mg of SO ₄ ²⁻ /10-ml portion on incubation days:								
	0	2	4	8	10	14	18	24	28
<i>Thiobacillus concretivorous</i>	3.4	10.5	17.5	25.6	35.7	39.2	47.3	47.0	50.2
<i>T. thiooxidans</i>	3.7	14.1	19.2	26.3	30.6	36.7	41.9	46.9	51.7
<i>T. thiooxidans</i> + shale	3.2	12.7	16.5	24.9	35.2	37.4	42.7	45.2	52.4
<i>T. concretivorous</i> + shale	3.3	14.2	16.8	27.2	36.1	35.9	44.3	46.1	52.8

^a Liter quantities of inorganic salt medium with 10 g of sulfur per liter were incubated at room temperature without shaking. Samples containing shale contained 20 g of mesh shale (no. 16 to 42) per liter. Portions of 10 ml were removed and the sulfate was determined gravimetrically after being precipitated as BaSO₄.

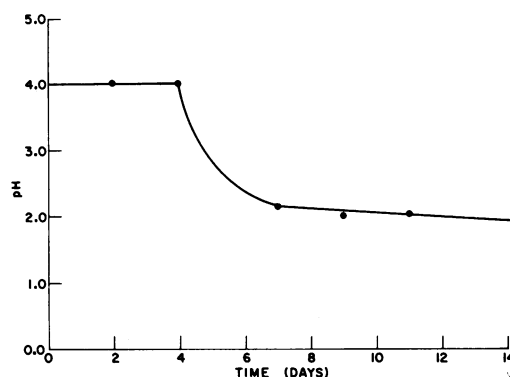


FIG. 3. Change in pH of the effluent from columns of crushed shale being leached with *T. thiooxidans* culture at an initial pH of 1.7 to 1.9. The columns contained 50 g of mesh shale (no. 16 to no. 42). The leaching solution was percolated down through the shale at the rate of 1 liter per day. The shale was continuously submerged in leaching solution.

the column was buffered initially at pH 4.0 by carbonates solubilized from the shale sample. Reaction of the carbonate portion of the shale was indicated by vigorous evolution of gas (CO₂). The rate of weight loss (Fig. 4) corresponded to the loss of buffering by the shale (Fig. 3). Evolution of visible gas ceased at about 5 days.

Ferrous ion derived from the pyrites appeared in the leaching column effluent (Fig. 5). The amount of ferrous ion extracted from shale reached a peak at 2 to 3 days and decreased slowly for the course of the experiment. Qualitative tests for ferric ion using potassium thiocyanate gave negative results.

Duplicate experiments showed that shale leached with *T. thiooxidans* lost 39.30% in weight and shale leached with *T. concretivorous* lost 39.00% in weight (Table 2).

The results of carbonate determination on native shale and on bioleached shale samples are presented in Table 3. The shale sample used

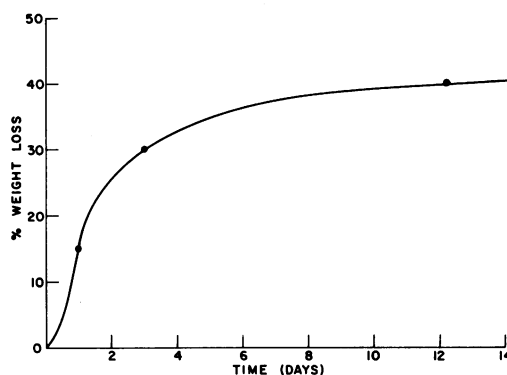


FIG. 4. Percentage of weight loss of shale recovered after leaching with *T. thiooxidans* culture solution will have an initial pH of 1.7 to 1.9. Leaching was continued for the length of time indicated before the shale was dried and weighed.

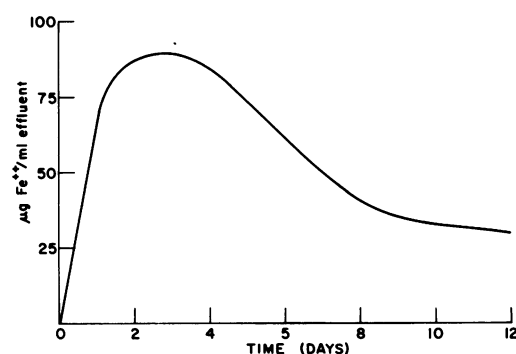


FIG. 5. Fe²⁺ content of shale column. Ferrous ion was determined spectrophotometrically after reaction with 1,10-phenanthroline. The shale sample size was 50 g of Tyler standard screen mesh (no. 16 to no. 42). *Thiobacillus thiooxidans* culture at an initial pH of 1.7 to 1.9 was used to leach the shale at a flow rate of 1 liter per day.

for the determination of carbonate carbon after bioleaching had lost 36.5% in weight. Removal of 97.0% of the carbonate was found.

TABLE 2. Observed percent weight loss of shale leached with cultures of sulfur oxidizers^a

No.	Sample	% Wt loss	Avg wt loss (g)
1	<i>Thiobacillus thiooxidans</i>	40.40	39.30
2	<i>T. thiooxidans</i>	38.20	
3	<i>T. concretivorous</i>	38.12	
4	<i>T. concretivorous</i>	39.87	

^a 50 g of shale leached for 14 days; flow rate of 1 liter per day.

TABLE 3. Comparison of carbonate content of unleached shale and bioleached shale

No.	Sample	Carbonate (%)	Corrected carbonate loss (%)
1	Unleached shale	20.09 ^a	95.8
2	Bioleached shale	1.35 ^b	

^a 4 Determinations, mean value.

^b 2 Determinations, mean value.

The effect of the acidity of the leaching solution is shown in Table 4. The organic acids at 0.1 N concentration were less effective in solubilizing the shale sample than 0.1 N H₂SO₄. The total percentage of shale solubilized by sulfuric acid was not increased by increasing the acid concentration from 0.1 to 1.0 N in the volume used.

Addition of 0.01% ethylenediaminetetraacetate to 0.1 N sulfuric acid was not effective in increasing the weight loss of leached shale.

Thiobacillus species are known to produce a wetting agent, phosphatidyl inositol (6). Shale was leached with 0.1 N sulfuric acid with 0.001% Triton X-100. There was no augmentation in weight loss as a result of adding a wetting agent to a 0.1 N H₂SO₄ leaching solution.

Shale samples were leached with *T. thiooxidans* culture for 1, 3, 12, and 14 days. The shale was recovered, dried, and weighed. The rate of weight loss is shown in Fig. 4. Weight loss occurred at the maximum rate during the early contact period and asymptotically decreased in rate.

DISCUSSION

The results of these experiments indicate that dolomite present in shale can be solubilized by the action of sulfur-oxidizing bacteria in a way similar to that used to extract uranium (5) molybdenum, chromium, and copper from low-grade ores (1). Solubilization of uranium ore has been mediated by the *Thiobacillus-Ferrobacil-*

lus group, which oxidizes pyrite to produce ferric ion and sulfuric acid (8). In these experiments dolomite has been solubilized by the formation of sulfuric acid from added sulfur, leaving a complex of the silica and kerogen. This complex is so tightly bound that although it can be burned to a silica slag, only the bituminous portion can be extracted with solvents.

Partial solubilization of minerals and rocks has been attributed to salts of 2-ketogluconic acid (11), inorganic acids (9, 11), organic acids (9), and phosphoglyceric acid (10). The mechanism has been shown to be due, for the most part, to acid-soluble constituents and the chelating action of organic acids (11).

The two sulfur-oxidizing organisms *T. concretivorous* and *T. thiooxidans* multiply quite rapidly for chemolithotrophs and almost equally well in still culture in mineral salt medium containing sulfur (Fig. 1). We decided to use *T. thiooxidans* in most of the experiments because more literature is available on this organism. *T. thiooxidans* decreased the pH of the mineral salt medium from 3.5 to approximately 1.9 in the lightly buffered medium. However, in equivalent flasks in which 20 g of shale per liter was present, the pH decreased to approximately 2.2 and then increased again (Fig. 2) as the H₂SO₄ that was being formed continually reacted with the dolomite. The reason for the original decrease in pH possibly lies in the fact that the sulfuric acid produced by the organisms could not attack the hydrophobic shale until sufficient wetting agent (6) produced by the organism permitted contact.

In the bioleaching experiments in which 50 g of shale was continuously leached with 14 liters of a 6-week culture of *T. thiooxidans* at the rate of 1 liter per day (Fig. 3), there was no change in

TABLE 4. Observed percent weight loss of shale leached with acid or acid plus an additive^a

No.	Sample	% Wt loss
1	0.1 N oxalic acid	23.34
2	0.1 N citric acid	28.78
3	0.1 N H ₂ SO ₄	38.45
4	1.0 N H ₂ SO ₄	39.12
5	0.1 N H ₂ SO ₄ + 0.01% EDTA ^b	37.92
6	0.1 N H ₂ SO ₄ + 0.001% Triton X-100	38.24

^a 50 g of shale leached for 14 days; flow rate of 1 liter per day.

^b EDTA, Ethylenediaminetetraacetate.

pH for the first 5 days due to the buffering action of the readily available $\text{MgCa}(\text{CO}_3)_2$. After this time, the pH dropped, rapidly approaching the pH of the original culture. A similar curve is obtained by equivalent leaching with 0.1 N H_2SO_4 .

The loss in weight of shale in the perfusion column (Fig. 4) parallels what would be expected from the preceding pH curves of the effluent. Although 30% loss in weight occurs within 3 days, there is still sufficient $\text{MgCa}(\text{CO}_3)_2$ present to buffer the pH of the effluent, and it is not until the day 5 or 6 in the experiments that a rapid decrease in pH occurred, although this represented a lesser proportional decrease in weight.

It was desired to obtain another indicator of speed of the dissolution of the shale, and one existed in the ability of acid formed to dissolve iron-containing minerals. The iron measured as ferrous iron was rapidly released from the shale (Fig. 5). The maximum peak was obtained at 2 or 3 days before the time that the significant decrease in pH occurred.

The shale residue after extraction of dolomite through the action of *Thiobacillus* is much more porous. As a result, several possibilities of using the material exist. For example, it is possible to use fractional distillation much more economically than with the original shale because 40 to 45% of the original weight has been removed. In addition, the possibility exists that the kerogen may be extracted by breaking the complex linkages by means of microorganisms or a microbiological method of splitting the silica linkages may be attained.

ACKNOWLEDGMENTS

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